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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Krieg
Serial No:	09/337,893
Filed:	June 21, 1999
For:	METHODS OF REDIRECTING AN IMMUNE RESPONSE USING IMMUNOSTIMULATORY OLIGONUCLEOTIDES
Examiner:	J. Martinell
Art Unit:	1633

ASSISTANT COMMISSIONER FOR PATENTS
WASHINGTON, D.C. 20231

CERTIFICATE OF MAILING UNDER 37 C.F.R. §1.8(a)

The undersigned hereby certifies that this document is being placed in the United States mail with first-class postage attached, addressed to, Assistant Commissioner for Patents, Washington, D.C. 20231, on July 23, 2001.

MaryDilys Anderson

Sir:

DECLARATION OF DR. ARTHUR KRIEG UNDER 37 CFR §1.32

I, Dr. Arthur M. Krieg, declare as follows:

1. I make this Declaration in support of U.S. Serial No. 09/337,893 on which I am named as an inventor.

2. I am the Chief Scientific Officer of Coley Pharmaceutical Group, Inc. Wellesley, MA. I have been performing research on CpG immunostimulatory nucleic acids for several years.

3. Experiments performed by myself as well as many investigators around the world, have demonstrated that CpG oligonucleotides administered alone or in combination with other drugs are effective when delivered by a variety of administration routes as well as into a variety of different subjects. I present a sampling of these findings below.

4. The results of a human clinical trial involving the administration of CpG oligonucleotides in conjunction with an antigen to healthy human volunteers have been released. The clinical study was performed under the direction of Dr. Heather Davis, a principal investigator at the Loeb Health Research Institute at the Ottawa Hospital in Ottawa, Canada, and Director of Vaccine Development of Coley Pharmaceutical Group, Canada. The trial was a Phase I study of Engerix-B hepatitis B vaccine with or without CpG oligonucleotide, referred to as 7909, which was double-blinded, randomized, rising dose, controlled. The results shown in the attached Exhibit 1 demonstrate that CpG oligonucleotides when administered in conjunction

with antigen to humans dramatically improves the antigen-specific immune response. Two weeks after treatment with the antigen alone or antigen plus CpG oligonucleotide, 0% of the subjects receiving antigen without CpG had seroconverted, whereas 83% of the human subjects receiving CpG oligonucleotides plus antigen had seroconverted. By six-eight weeks following the initial administration, and after a single boost, 100% of the human subjects receiving antigen plus CpG oligonucleotide had seroconverted. The data revealed that CpG oligonucleotides significantly improved response rates, markedly improved antibody titers, and provided earlier seroprotection (even when compared with a competing immune stimulant, MPL). No serious adverse events were reported. The results of the trial were consistent with those obtained in various preclinical animal studies and *in vitro* cell culture assays. In this clinical study, the antigen and CpG oligonucleotides were administered parenterally.

5. Another study was performed on a population of non-human primates which were hyporesponsive to hepatitis B vaccine, Engerix-B ®. The animals were vaccinated with CpG oligonucleotides in combination with Engerix-B. The addition of the CpG oligonucleotides to the vaccination program markedly increased seroconversion and seroprotection rates and greatly enhanced antibody titers, as shown in the graph of Exhibit 2. After two doses, 100% of animals receiving CpG oligonucleotide plus Energerix-B had protective levels of antibodies compared to only 8% with the commercial vaccine alone. The CpG oligonucleotide and vaccine were delivered parenterally to the non-human primates.

6. Additionally two papers, attached hereto as Exhibits 3 and 4, describe immunization of Aotus monkeys and orangutans with CpG and malaria vaccine or hepatitis B vaccine respectively.

7. CpG oligonucleotides either alone, or with antigen, have been delivered orally, nasally, and rectally to produce both mucosal and systemic immune responses. The data presented in Exhibit 5 demonstrate that oral delivery of oligonucleotides in combination with a hepatitis B antigen. HbsAg induced systemic and mucosal antibody production. Additionally, IgA was induced at remote mucosal surfaces as evidenced by the presence of IgA in lung washes, feces, gut wash, vaginal wash and saliva.

8. Systemic and mucosal immunization were also observed when CpG oligonucleotides were delivered intranasally with influenza virus or hepatitis B antigens as demonstrated in Moldoveanu, Z. et al., *Vaccine*, 16:1216-24 (1998) and McCluskie, M.J. and Davis, H.L., *J. Immunol.*, 161:4463-6 (1998), attached hereto as Exhibit 6 and 7, respectively.

9. CpG oligonucleotides were also demonstrated to produce an immune response when delivered intra-rectally, either alone or with formalin-inactivated tetanus toxoid (TT) antigen. The results are shown in Exhibit 8. Delivery of the TT by the intra-rectal route resulted in 0 out of 5 mice responding in the absence of CpG oligonucleotide and 8 out of 10 mice responding in the presence of CpG oligonucleotide.

10. I, Dr. Arthur Krieg, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of this document and any patent which may issue from the above-identified patent application.

Date: July 23, 2001

By: _____
Dr. Arthur Krieg